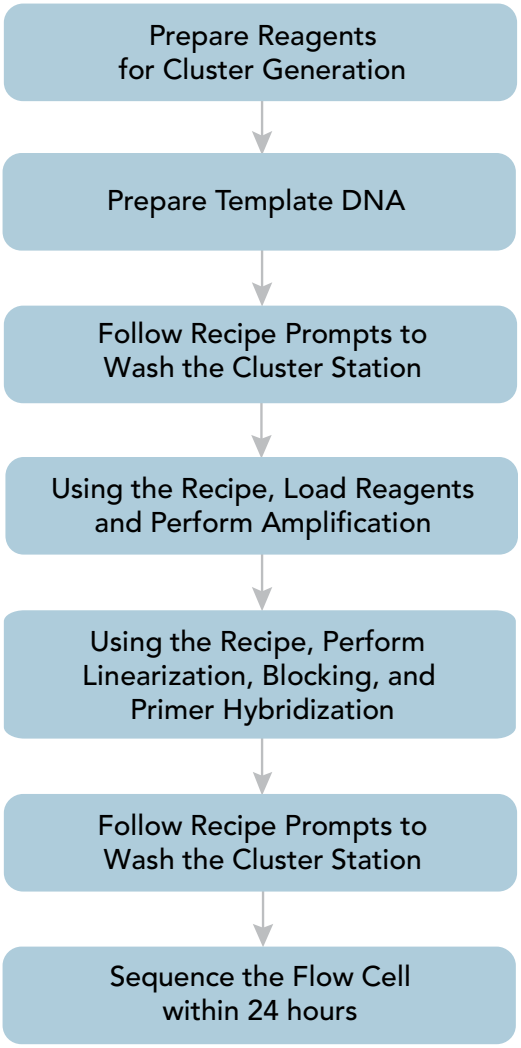
Paired-End Cluster Generation on the Cluster Station

Experienced User Card

This experienced user protocol explains how to perform paired-end cluster generation on the Cluster Station.

Allow approximately six hours to complete a cluster generation run.



Preparing for Cluster Generation

This section explains how to prepare reagents for amplification, linearization, blocking, and primer hybridization, and how to denature and dilute the DNA template.

Use reagents provided in the Paired-End Cluster Generation Kit v4 with the following protocol.

Prepare Reagents for Amplification

Thaw Reagents

[ ] **1.** Thaw HT1, HT2, HFE, AT1, APM1, AMX1, LMX1, BMX, HP1, HP3 at room temperature, and then place AMX1, HFE, LMX1, and BMX on ice.

[ ] **2.** Record the lot numbers on the lab tracking form.

Prepare AMX1

[ ] **1.** Invert the container of AMX1 five times and centrifuge at 1,000 rpm for one minute.

[ ] **2.** Label the container of AMX1 "Reagent #1" and then set aside on ice

Prepare HT2

[ ] **1.** Invert the container of HT2 five times, centrifuge at 1,000 rpm for one minute, and then label "Reagent #3."

[ ] **2.** Label an eight-tube strip "C" and pipette 100 µl of HT2 into each tube.

[ ] **3.** Label an eight-tube strip "G" and pipette 100 µl of HT2 into each tube.

Prepare AT1

[ ] **1.** Invert the container of AT1 five times, centrifuge at 1,000 rpm for one minute, and then label "Reagent #9."

Prepare APM1

[ ] **1.** Invert the container of APM1 five times and label "Reagent 11."

[ ] **2.** Label an eight-tube strip "D" and pipette 100 µl of APM1 into each tube of the eight-tube strip.

Prepare HT1

[ ] **1.** Invert the container of HT1 five times, centrifuge at 1,000 rpm for one minute, and then label "Reagent #12."

[ ] **2.** Label an eight-tube strip "A" and pipette 140 µl of HT1 into each tube.

Prepare HFE (1X Phusion™ Master Mix, Finnzymes Oy)

[ ] **1.** Invert the container of HFE several times and pulse centrifuge the reagent.

[ ] **2.** Label an eight-tube strip "E" and pipette 120 µl of HFE into each tube, and then set aside on ice.

Prepare HP3

[ ] **1.** Invert the container of HP3 five times to mix and then pulse centrifuge.

[ ] **2.** Transfer 1,425 µl of PW1 into a 1.5 ml microcentrifuge tube and add 75 µl of HP3, and then briefly pulse centrifuge.

[ ] **3.** Label an eight-tube strip "F" and pipette 100 µl of diluted HP3 into each tube.

Prepare Reagents for Lin/Block/Hyb

Prepare BMX

[ ] 1. Invert the container of BMX five times and then label "Reagent #4."

[ ] 2. Pulse centrifuge the reagent and then set aside on ice.

Prepare HP1

[ ] 1. Invert the container of HP1 five times and then label "Reagent #7."

[ ] 2. Pulse centrifuge the reagent and then set aside on ice.

Prepare LMX1

[ ] 1. Invert the container of LMX1 five times and then label "Reagent #14."

[ ] 2. Pulse centrifuge the reagent and then set aside on ice.

Prepare HP3

[ ] 1. Invert the container of HP3 five times to mix and then pulse centrifuge.

[ ] 2. Transfer 1,425 µl of PW1 into a 1.5 ml microcentrifuge tube and add 75 µl of HP3, and then briefly pulse centrifuge.

[ ] 3. Label the container "Reagent #17."

Prepare DNA Template

[ ] 1. Combine the following volumes of template DNA, Tris-Cl, and HP3:

- 10 nM Template DNA (2 µl)
- Tris-Cl 10 mM, pH 8.5 (17 µl)
- HP3 (2 N NaOH) (1 µl)

[ ] 2. Vortex briefly to mix and then pulse centrifuge. Incubate for 5 minutes at room temperature.

[ ] 3. Dilute the DNA to a final concentration of 6–8 pM with pre-chilled HT1 to a total volume of 1 ml.

[ ] 4. Invert several times to mix the template solution, and then pulse centrifuge.

[ ] 5. Label the tube strip "B" and number the tubes 1–8.

[ ] 6. Dispense 120 µl of the Illumina control DNA library into tube 4 of an eight-tube strip.

[ ] 7. Add 120 µl of diluted, denatured sample DNA template into the remaining tubes of an eight-tube strip.

[ ] 8. Record each sample position and concentration on the lab tracking form.

[ ] 9. Set aside on ice.

Performing Cluster Generation

This section describes the steps required to perform cluster generation on the Cluster Station using a one-step recipe.

Use a version 7 cluster generation recipe with the following protocol.

Perform Cluster Generation

[ ] 1. Weigh each of the prepared reagents and record the weight on the lab tracking form.

[ ] 2. Select **File** | **Open Recipe** and open the following recipe, and then click **Start**:

PE_Amplification_Linearization_CombinedBlocking_PrimerHyb_v7.0.xml

[ ] 3. Follow the prompts in the recipe to perform amplification:

- **[ ] a.** Attach the washing bridge and load water in positions 1, 3, 4, 7, 9, 11, 12, 14, and 17.
- **[ ] b.** When washing of lines is finished, remove water from reagent positions. Click **OK** to proceed to priming of the air gap.
- **[ ] c.** When the air gap is primed, load reagents in positions 1, 3, 4, 7, 9, 11, 12, 14, and 17. Click **OK** to proceed.
- **[ ] d.** Load the flow cell, hybridization manifold, and tube strip holder.
- **[ ] e.** Load tube strip "A" (Hybridization Buffer) oriented such that tube #1 is toward the back of the tube strip holder.
- **[ ] f.** Replace tube strip "A" with tube strip "B" (Template Mix).
- **[ ] g.** Replace tube strip "B" with tube strip "C" (HT2, Wash Buffer).
- **[ ] h.** Replace tube strip "C" with tube strip "D" (APM1, AMX1 Premix).
- **[ ] i.** Replace tube strip "D" with tube strip "E" (HFE, 1X Phusion Master Mix, Finnzymes Oy).
- **[ ] j.** *Promptly* replace tube strip "E" with tube strip "F" (HP3, 0.1 N NaOH).
- **[ ] k.** Replace tube strip "F" with tube strip "G" (HT2, Wash Buffer).
- **[ ] l.** Replace the hybridization manifold with the amplification manifold. Click **OK** and check for proper flow.

[ ] 4. When prompted, remove the flow cell.

[ ] 5. Weigh each of the reagents and record the weight on the lab tracking form.

[ ] 6. Resume the recipe and follow the prompts to attach the washing bridge.

[ ] 7. Load water in positions 3, 4, 7, 12, 14, and 17, and then proceed to washing the lines.

[ ] 8. Sequence the flow cell within 24 hours.